REMARKS

Claims 1-82 are pending and claims 83-86 are added herein. Claims 5, 19-22, 23, 30-52, 54-71, 73-77, 79-82 are withdrawn from consideration, pursuant to a Restriction of Invention requirement. Upon entry of the foregoing amendment, claim 78 will be amended, claims 83-86 will be added, and those claims which are withdrawn from consideration (*i.e.* claims 19-22, 23, 30-52, 54-71, 73-77, and 79-82) will be canceled without prejudice.

The newly presented and amended claims are supported by the specification as originally filed. Claim 83 is supported at, *e.g.*, page 5, lines 1-3. Claim 84 finds support at page 6, line 26, for example. Claim 85 is supported, *e.g.*, at page 6, lines 26-28, and page 15, lines 4-9. There is support for claim 86 at, *inter alia*, page 14, line 37 thorough page 15, line 2.

Applicants affirm the election of the invention of Group I and claims 1-4, 6-18, 23, 25-29, 53, 72, 78 and 83-86 are submitted for examination on the merits. Of course, Applicants reserve the right to file one or more divisional applications directed to the canceled claims.

I. Rejection Under 35 U.S.C. § 112

Applicants note with appreciation that each of the claims are free of the prior art. The sole rejection in this case is one of enablement, pursuant to 35 U.S.C. § 112, first paragraph.

The rejected claims are directed to a single-chain multiple antigen-binding molecule. This molecule comprises (a) a variable domain of a heavy chain of an immunoglobulin (VH) with a first specificity (A) (or functional parts thereof); (b) a variable domain of a light chain of an immunoglobulin (VL) with a second specificity (B) (or functional parts thereof); (c) a variable domain of a heavy chain of an immunoglobulin (VH) with the specificity (B) (or functional parts thereof); and (d) a variable domain of a light chain of an immunoglobulin (VL) with the specificity (A) (or functional parts thereof), wherein the VH and VL domains are connected in the form of a VH-VL construct or VL-VH construct, and wherein the two VH-VL constructs are connected via a peptide (P).

The Examiner has stated that the specification does not enable one of ordinary skill in the art to make and use the claimed invention. According, claims 1-4, 6-18, 23, 25-29, 53, 72, and 78 have been rejected under 35 U.S.C. § 112, first paragraph.

This rejection appears to be premised on the observation that the specification only contains working examples directed to a single chain binding molecule that is capable of binding both CEA and *E. coli* β-galactosidase. However, Applicants object to the Examiner's apparent attempt to limit the scope of the claims to specific examples set forth in the specification. Examples are not required to establish sufficient support for claims under 35 U.S.C. § 112, first paragraph, so long as the invention may be practiced without undue experimentation. *In re Borkowski*, 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970). For the reasons which follow, Applicants will demonstrate that, when considering the nature of these rejections and the teachings of the art as a whole, Applicants' choice to include only these particular working examples in the Application is not a sufficient basis to reject the claims.

a. making a single chain binding molecule with dual specificity,* e.g., *for (1) the cell membrane of a target cell and (2) a vector.

The Examiner first argues that the specification does not provide an enabling disclosure for *making* a single chain binding molecule with dual specificity for the cell membrane of a target cell and a vector (*see* Office Action at page 4, lines 10-11). However, **Example 1**, for instance, provides more than enough evidence that the disclosure enables one of ordinary skill in the art to

prepare such a binding molecule. To this end, Example 1 illustrates that Applicants constructed a single chain binding molecule that is capable of binding CEA and β-galactosidase, comprising: VH_1-anti-CEA, VL_2-anti-β-galactosidase, VH_2-anti-β-galactosidase, and VL_1-anti-CEA (*see* specification at page 27, line 23 through page 29, line 31; *see also* Figure 1).

At the time of the invention, a skilled artisan would have been able to replace the VH and VL specificities with those for a cell membrane of a target cell and a vector, respectively. Two methods conventionally are used to clone VH and VL domains. The first method recruits established hybridoma-secreting antibodies, using the desired specificity as the starting point. The second approach uses antibody phage display libraries to isolate specific antibody fragments (*e.g.*, ScFv, Fab).

According to the first method, RNA is isolated from the hybridoma and is reversed transcribed into cDNA, using oligo-dT or random hexamer primers. The VH- and VL-encoding DNA is amplified, *e.g.*, by PCR, using a primer specific for the variable domains, as described by Dubel *et al.*, *J. Immunol. Meth.* 175:89-95 (1994), and Krebber *et al.*, *J. Immunol. Meth.* 201:35-55 (1997).

In the second approach, such phage display libraries either are generated from immunized (*i.e.*, immune library) or non immunized (*i.e.*, naïve library)

donors, or are generated synthetically by genetic engineering, as described by Winter *et al., Annu. Rev. Immunol.* 12:433-55 (1994), and Vaughan *et al., Nature Biotechnol.* 14:309-14 (1996). Then, specific antibodies are selected, using, for example, immobilized purified polypeptides (Nissim *et al., J. Immunol. Meth. EMBO J.* 13:692-98 (1994)), whole cells (de Kruif *et al., J. Mol Biol.* 248:97-105; (1995)), or tissue sections (Tordsson *et al., J. Immunol. Meth.* 210:11-23 (1997)).

Each method has been successfully applied in various contexts. For example, antibodies have been isolated against DNA, as shown by Jang *et al., Mol. Immunol.* 35: 1207-17 (1998). Antibodies have been isolated against viral coat proteins, as disclosed by Watkins *et al., Gene Ther.* 4: 1004-12 (1996). Also, antiobodies have been isolated against a large panel of different cell surface antigens, such as CEA (Osbourn *et al., Immunotech.* 2:181-96 (1996)) and HMW-MAA (Noronha *et al., J. Immunol.* 161:2968-76 (1998)).

The overall success of recombinant antibody production shows that, based on Applicants' submission of Example 1, the guidance provided in the specification and known teachings in the art, one of ordinary skill in the art could—by conventional means—construct other single chain binding molecules according to the claimed invention, *e.g.,* those which have a specificity for both a target cell and a vector.

Besides clarifying that the foregoing teachings were well known in the art as of the filing date, Applicants also respectfully point out that the specification, itself, provides further guidance and suggestions as to how to choose a particular binding domain (depending on the desired target), and implement said domain into a binding molecule according to the claimed invention. Indeed, the specification references a myriad of immunoglobulins that are suitable for use in the claimed invention. For instance, the instant specification at page 10, lines 10-14 states,

> Examples of target cell-specific ligands, of membrane structures on the target cell, of target-cell0specific ligands and of gene construct-specific ligands which are derived from immunoglobulins, *i.e.* comprise VH and VL domains, as well as of peptides with a fusogenic property are described in detail in DE 19649645.4.

The specification (at page 27, lines 17-19) incorporates this document by reference.

DE 19649645.4, although written in German, is virtually identical to EP 0846772, which is written in English. Thus, the disclosure of EP 0846772 corresponds to that of DE 19649645.4 and provides examples of immunoglobulins having binding specificities against various constructs, including modified DNA, unmodified DNA and a viral coat protein.

Examples of monoclonal antibodies that have a binding specificity against modified DNA are listed at page 10, lines 24-29 of DE 19649645.4 (page 12, lines 12-26 of EP 0846772). These include, *inter alia*, construct-specific ligands against a modified DNA epitope, such as: methylated DNA, N^7-ethylguanine, N^5-methyl-N^5-formyl-2,5,6-triamino-4-hydroxypyrimidine, O^4-methyl-2'-deoxythymidine, O^4-ethyl-2'-deoxythymidine, methylated N^6 of adenine, addition products of melphalan and DNA, and anthracyclines.

These supporting references also list antibodies directed against one or more epitopes of a viral coat protein. Such viral proteins include protein sp70 and adenovirus (Page 10, line 44 through page 11, line 7 of DE 19649645.4; page 12, line 49 through page 13, line 40 of EP 0846772).

Examples of target cell-specific ligands are listed, *e.g.*, at page 5, line 43 through page 8, line 44 of DE 19649645.4 (page 5, line 17 through page 9, line 62 of EP 0846772). This include, *inter alia,* ligands directed against membrane structures of endothelial cells, such as TGF-β or VEGF receptors; against membrane structures of lymphocytes and macrophages, such as IL-1, IL-2, EGF, and IGF; aginst membrane structures of smooth muscle cells, *e.g.,* against actin, angiotensin II receptors, and TGF; against receptors of hematopoietic cells, such as stem cell factor receptor, IL-1 receptor (type I & II), and IL-3 R-α; against

membrane structures of liver cells, including growth factors such as cytokines, EGF, TGF, FGF and PDGF; and against membrane structures of glial cells, including N-CAM and insulin-like growth factor; against membrane structures on tumor cells, *e.g.,* sialyl Lewis, peptides recognized by T-cells, peptides expressed by oncogenes, gangliosides (*e.g.,* GD3), blood group antigens and precursors thereof.

Accordingly, the specification alludes to a myriad of molecules, cell membrane fragments, cells and viruses which may be used as "starting material" for the construction of single chain multiple antigen-binding molecules according to the claimed invention. As such, the Examiner's reliance on *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed. Cir. 1986) is inapposite (court finding enablement requirement not satisfied because specification discloses[, *inter alia,*] any specific starting material).

The Examiner also has observed that the Application does not purport to incorporate-by-reference any references, such as the foregoing, in the context of making a single chain binding molecule (*see* Office Action at page 4, lines 16-17). However, page 27, lines 15-19 of the specification incorporates-by-reference each reference mentioned therein.

Regardless, Applicants vigorously point out that incorporation-by-reference is not the standard for adjudging the enablement requirement. Rather, the issue is whether a skilled worker could make and use the claimed invention, based on the teaching of the specification and the available knowledge at the effective filing date. Accordingly, the specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skillled and already available to the public. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991).

In view of the exhaustive list of references Applicants have supplied (in addition to pointing out the teachings of, and successes reported by these references), Applicants respectfully urge that a skilled worker could make the claimed invention. Moreover, Applicants cogently have indicated that, in the context of the "species" elected pursuant to the restriction requirement, the specification is not limited to the statement that "it is advantageous for the second specificity (B) to be against a vector...," as the Examiner suggests (Office Action, *id.* at lines 18-20). Accordingly, the Examiner respectfully is urged to withdraw this rejection.

b. affinity and avidity for a single chain binding molecule with dual specificity

The Examiner also appears to base the enablement rejection on the specification's alleged failure to provide sufficient guidance as to the affinity and avidity of the vector binding the VH and VL domains (*see id.* at page 5, lines 19-22). Applicants traverse this rejection, however, for the following reasons.

As far as affinity and avidity is concerned, Applicants respectfully urge that one of ordinary skill in the art would expect that—depending on the chosen starting material—(1) the affinity and avidity of VH and VL domains of a single chain multiple antigen-binding molecule would vary within the range commensurate with normal (*e.g.,* monoclonal) antibodies and (2) a skilled worker could make use of VH and VL domains having an affinity and avidity within this range. For instance, Watkins *et al., Nat. Biotechn.* 14:309-14 (1996) have successfully have employed various recombinant antibodies against viral vectors or cell surface antigens, which have a nano-molar affinity. Since avidity strongly is influenced by the number of antibody molecules bound per vector or effector molecule, the binding of several antibodies to a viral vector likely would result in an increased avidity. Thus, there is no objective reason to doubt that the teachings in the specification enable a skilled artisan to practice the claimed invention.

c. physical or biological constraints affecting uptake of a ligated vector by a target cell.

The Examiner also states that the specification lacks sufficient guidance as to the physical or biological constraints affecting uptake of a ligated vector by a target cell (*see id.* at lines 24-25). Again, this rejection appears to be that the Specification does not specifically include a working example of this embodiment (*see id.* at page 6, lines 8-10).

However, Applicants note that the compliance with the enablement requirement does not obligate Applicants to actually carry out that which would have been routine to one of ordinary skill in the art (*see* MPEP § 2164.02). In this context, Applicants point out that the structure and characteristics of the claimed multi-antigen binding molecules make them conducive to, if not ideal for, cell targeting and cellular internalization. Indeed, the modular structure of ScMAB would allow it to combine, *e.g.*, a vector-specific binding site with a target cell-specific binding site that recognizes a cell surface antigen that mediates internalization after binding.

To buttress Applicants' position, Applicants report herewith of the successful infection of a target-cell by a vector that is bound to a claimed molecule. In particular, Applicants confirmed that a single chain multiple-antigen

binding molecule, bound to an adenovirus capside, successfully targeted an endothelial surface protein endoglin. This resulted in endothelial cells uptaking the "multiple-antigen binding molecule—adenovirus" complex, followed by adenoviral vetor infection of the targeted cell. This success is reported by: Nettelbeck DM, Miller DW, Jerome V, Zuzarte M, Watkins SJ, Hawkins RE, Muller R, and Kontermann RE, *Mol. Ther.*, "Targeting of Adenovirus to Endothelial Cells by a Bispecific Single-Chain Diabody Directed against the Adenovirus Fiber Knob Domain and Human Endoglin (CD105)." 3(6):882-91 (2001). The underlined scientists are named inventors in the subject Application. Such post-filing data evinces that the specification complies with the enablement requirement in the instant Application.

Nonetheless, it is Applicants' position that—even absent further working examples and post-filing data—the specification, as filed, satisfies the enablement requirement, when viewed in conjunction with those teaching available to a skilled artisan at the time of filing. Along these lines, Applicants respectfully request the Examiner to keep in mind that, in most instances, a vector or plasmid is internalized either (a) directly by processes mediated, *e.g.*, by a cell membrane-targeting molecule or (b) indirectly, *e.g.*, by secondary interations between the vector and the target cell.

In above situation "(a)," Applicants earnestly submit that the mechanism involved in binding a single chain multiple antigen-binding molecule to its respective antigen(s) (*e.g.,* vectors, target cells, cell membrane receptors) is the same as, or similar to, that which is involved in conventional (*e.g.,* monoclonal) antibody-antigen, Fab-antigen and diabody-antigen interactions. In these situations, the binding molecules interact with the antigen at the VH and VL domains of the immunoglobulins, for facilitation of internalization.

For example, direct single chain Fv-induced internalization usually requires (a) binding to a cell surface receptor and (b) the induction of receptor-mediated processes that lead to internalization and endosomal release. Generally, this is caused either by receptor dimerization or by conformational changes of the receptor, both of which are induced by the binding of a ligand in order to mediate internalization (*see* Ullrich *et al. Cell* 61:203-212 (1990)).

In any event, one of ordinary skill in the art would recognize that an antigen (such as a cell surface receptor) can mediate uptake of an antibody, upon binding. Examples of receptors that behave in this way include the EGF receptor, described by Spaargaren, *et al., J. Biol. Chem.* 266:1733-39 (1991); erbB2, which is illustrated by Tagliabue *et al., Int. J. Cancer* 47:933-37 (1991); and the transferrin receptor, as reported by Lesley *et al., Expage Cell Res.* 182:215-33 (1989).

In view of the foregoing, it is Applicants' contention that a skilled worker reasonably would predict that the claimed multiple antigen-binding molecules would behave in a fashion similar to any immunoglobulin, given that the claimed molecules would be expected to interact with a target cell in the same (or similar) fahion to an immunoglobulin that binds an antigen via VH and VL domains. In light of the well-documented successes in the art regarding the targeting of a vector to a cell membrane via an immunoglobulin (followed by internalization), Applicants urge that there is no objective reason to doubt that a multiple antigen-binding molecule, as claimed, would interact with a target cell in such a way to allow for internalization of, *e.g.,* a vector or plasmid.

Moreover, there currently are no known insurmountable limitations concerning the size of antibody-vector complexes that may be internalized. For instance, small DNA complexes comprising DNA and a fusion protein which contain an anti-erbB2 scFv, a DNA-binding domain and the *Pseudomonas* exotoxin-A internalization domain are conventionally used, as shown by Uherek *et al., J. Biol. Chem.* 273:8835-41 (1998). Towards the other end of the spectrum, large viruses (*e.g.,* adenoviruses) successfully can be complexed with bispecific antibodies for cellular internalization, as evidenced by Douglas *et al., Nat. Biotechn.* 14:1574-78 (1996), and Wickham *et al., J. Virol.* 70:6831-38 (1996).

Applicants additionally refer to the use of a fusogenic peptide to facilitate internalization of, *e.g.,* a vector. In this regard, Applicants refer to page 10, lines 13-14 of the specification, which explains that application DE 19649645.4 (English language counterpart: EP 0846772) describes fusogenic peptides in detail. Page 10, lines 30-64 of DE 19649645.4 (page 9, lines 14-29 of EP 0846772) lists, *inter alia,* polypeptides that comprise: the peptide GLFEALLELLESLWELLEA, as disclosed by Gottschalk *et al., Gene Ther.* 3:448 (1996); the peptide AALAEA[LAEA]$_4$LAAAAGC, reported by Wang *et al., Technol. Advances in Vector Sys. for Gene Ther.*, IBC Conference (May 6-7, 1996); and the peptide FAGVVLAGAALGVAAAAQI of the measles virus fusion protein, as shown by Yeagle *et al., Biochem. Biophys.* 1065:49 (1991). Accordingly, the specification does, in fact, identify fusogenic peptides suitable for use in the claimed invention.

These reported successes provide further evidence that there is more than ample guidance in the art, as to not only the identity or physical characteristics of cells that are capable of taking up large molecular complexes, but also the identity of cellular receptors that can mediating uptake thereof. Thus, there is sufficient evidence that the claims are enabled by the specification and that the Examiner's comments should not offend the claims' presumption of validity.

d. enabling disclosure for the teatment or prophylaxis of disease, using a claimed molecule

The Examiner alleges that the specification does not provide an enabling disclosure for the treatment or propylaxis of disease by administering [to a subject] a single chain binding molecule of the invention (*see* Office Action at page 7, lines 1-4). Applicants first point out that claims 1-4, 6-18, 23, 25-29, 53, 72, and 83-85 are directed to a composition. Accordingly, any enabled use, *e.g.,* an *in vitro* use, is sufficient to satisfy the enablement requirement (*see* MPEP s 2164.01(c) (if any use is enabled when multiple uses are disclosed [for a compound or composition claim], the application is enabling for the claimed invention")).

As Applicant's have indicated herein, the specification teaches a skilled worker how to both make and use the claimed invention, in accordance with 35 U.S.C. § 112, first paragraph. For instance, the overall success with using recombinant immunoglobulins, such as Fab's and scFv's, for cellular internalization, would be indicative of success in using the claimed invention.

Moreover, Nettelbeck *et al., id.,* provides actual data that a single chain multiple-antigen binding molecule, bound to an adenovirus capside, successfully targeted an endothelial surface protein endoglin. Accordingly, claims 1-4, 6-18,

23, 25-29, 53, 72, and 83-85 meet the standard of enablement and the Examiner respectfully is requested to withdraw the rejection.

Applicants also contend that claims 78 and 86 (methods of using the claimed invention to prevent or treat disease) satisfy the enablement requirement. Applicants respectfully direct the Examiner to page 14, line 36 through page 15, line 2 of the specification. This portion discloses methods of administering a desired nucleic acid construct to a subject, including local administration to the patient directly, administration into a body cavity, into an organ, into the circulatory system, subcutaneously or intramuscularly. Furthermore, the specification describes multiple components, *e.g.,* promoters activators (*see* specification at pages 15-19), target cell specific ligands (*see* cited above-cited portions of DE 19649645.4), and ligands directed against vectors (*see* specification at page 9, line 32 through page 10, line 8), such that a skilled worker would be able to design a single chain multi-antigen binding molecule that targets a particular cell of choice, followed by internalization of, for example, a nucleic acid construct contained in a vector.

In addition to referencing the teachings in the specification and those that were available at the time of filing, Applicants also point out that Nettelbeck *et al., id.*, provide strong support that claims 78 and 86 are enabled by the specification.

By using the teachings of the specification, Nettlebeck *et al.* were able target a cell, using a single chain multi-antigen binding molecule bound to a adenovirus capsid, said adenovirus being taken up by the targeted cell. Accordingly, a withdrawal of the rejection is requested.

II. Miscellaneous

On a more formal note, the Examiner questions whether a vector is bound to the single chain binding molecule *in vitro* (*i.e.* pre-bound) or *in vivo*. The invention allows for either or both regimens. For instance, it is possible to mix the vector and binding molecule before administration, as performed by Pelegrin *et al., Hum. Gene Ther.* 9:2165-75 (1998). One benefit to this approach, as indicated by Verma & Somia, *Nature* 389:239-42 (1997), is increased targeting efficiency, since it reduces vector "trapping" in highly vascularized organs, ushc as the liver. However, pretargeting strategies are also contemplated by the invention, including administering the targeting molecule to a subject, followed by an optional clearing step, and thereafter administering a vector or effector, which binds the binding molecule. Such an approach has been reported by Zhu *et al., N. Nucl. Med.* 39:65-76 (1998) and Klivenyi *et al., J. Nucl. Med.* 39:1769-76 (1998).

CONCLUSION

Applicants submit that the present claims are in condition for allowance, and respectfully request consideration to that effect. Should the Examiner have any questions regarding the present application or believe that further discussion will advance prosecution, the Examiner is invited to contact the undersigned at the number listed below.

Respectfully submitted,

July 18, 2001
Date

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MARKED UP COPY OF AMENDED CLAIMS

78. (Amended) A method for the ~~diagnosis,~~ prophylaxis or treatment of cancer, ~~autoimmune diseases, inflammatory diseases, disorders of the blood, disorders of the nervous system or infectious diseases using~~ comprising administering to a patient a single chain multiple binding molecule as claimed in claim 1.